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Repression of photoreactivation and dark repair of coliform bacteria by TiO_2-modified UV-C disinfection

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ABSTRACT

Photoreactivation and dark repair of damaged DNA can occur after some bacteria are disinfected with UV-C irradiation and thus reduce the disinfection efficiency. We have discovered that the provision of 1 mg/L titanium dioxide (TiO_2) in suspension during UV-C irradiation at normal disinfection doses repressed the photoreactivation and dark repair. The repressive effect was also observed when a TiO_2-coated plate was used during UV-C exposure but removed thereafter, indicating the repressive effect does not require the presence of TiO_2 in the post-UV event. The repressive effect was consistently observed with changes of bacteria species, temperature, salinities, UV sources, doses and intensities, and with/without nutrients. The repressive effect is likely associated with the additional radical attack during UV-TiO_2 irradiation and/or formation of a small amount of stable residual oxidants (primarily hydrogen peroxide). The TiO_2-modified UV-C disinfection represents an innovative means to disinfect water. It is particularly useful in situations where additions of residual chlorine or chloramines are not allowed or not possible. Nevertheless, the TiO_2 shall be properly included, by either using the optimum dose in suspension or immobilizing it onto a surface, since too little TiO_2 cannot provide the repression while too much TiO_2 in suspension gives a detrimental effect on the UV-C disinfection.

1. Introduction

The control of the transmission of infectious diseases from water benefits greatly from proper means of disinfection. Ultraviolet (UV) irradiation at a wavelength in the UV-C range (190–280 nm) has been being considered a promising alternative to chlorine for its better effectiveness in the inactivation of a broad range of pathogens including bacteria, protozoan and viruses [1]. UV irradiation is also superior to chlorine with regard to its ease of operation and its no formation of toxic chloro-organic by-products in water [2].

Efficiency of UV disinfection is commonly measured by monitoring the fraction of cells that remain replicable, and that are not necessarily dead. When pathogens are exposed to a sufficient amount of UV-C irradiation, pathogens are inactivated. The inactivation relies primarily on the photochemical induction of lesions in the genomic DNA of the organisms, and the major lesions are cis–syn cyclobutane pyrimidine dimers (CPD) [3]. The thymine–thymine dimer linkage prohibits DNA replication and thus stops reproduction of pathogens essential to infection. However, by the time the UV illumination is removed, some microorganisms particularly bacteria are known to be capable of repairing their damaged DNA in the presence or absence of visible light by mechanisms commonly referred to as photoreactivation and dark repair, respectively. Photoreactivation is a process where microorganisms utilize light in the wavelength range of 330–480 nm to activate a specific enzyme, photolyase, in order to split CPD to recover the damaged DNA [4]. Dark repair, also called nucleotide excision repair, requires coordination of over a dozen proteins to excise and repair the damaged DNA segment [5]. Rates of photoreactivation are commonly faster than those of dark repair and the extents of photoreactivation and dark repair vary among different species and strains and under different conditions [6,7]. Evidences have shown that *Escherichia coli* (*E. coli*) is capable of photoreactivation and dark repair and photoreactivation of *E. coli* can be repressed after medium pressure (MP) UV irradiation [8]. The repressed photoreactivation is suggested to be attributable to the reversible oxidative damage to photolyase by MP UV [9]. However, *Legionella pneumophila* has been reported to photoreactivate nearly completely after irradiation with either low pressure (LP) or MP UV [8]. Thus, these two repair phenomena may give potential health risks of infection when UV irradiation is used as a stand-alone pathogen control means. In drinking water production, a small quantity of chlorine is added to the UV irradiated water to stop the repair by providing residual protection. Dosing a small quantity of chlorine to water leads to some formation of chloro-organic by-products such as trihalomethanes and haloacetic acids. In some instances (e.g., sewage

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effluent discharge and aquacultural systems), it is inappropriate to add chlorine, since it is toxic to aquatic lives.

Since the discovery of photoinduced decomposition of water on titanium dioxide (TiO_2) electrodes, the development of TiO_2-based photocatalytic technologies has made tremendous advances. One application of TiO_2-based photocatalysts is in the environmental area where the catalysts are used to promote degradation of organic pollutants and/or disinfection of microorganisms. Such processes are commonly referred to as TiO_2-based photocatalytic oxidation/disinfection processes and the processes utilize lights at a wavelength in the UV-A (315–400 nm), UV-B (280–315 nm), or UV-C (190–280 nm) range to induce an electron to jump from the valence band to the conduction band to create the hole/electron pair [10]. The conduction band electron is available for electron transfer to reducible species, such as O_2 and H_2O_2, adsorbed on the TiO_2 surface to form hydroxyl radicals ($^{\bullet}OH$). The hole can react with water or hydroxide ions (OH^-) to form $^{\bullet}OH$ and other reactive oxygen species (ROS). These reactive radicals and oxygen species are generally considered responsible for chemical oxidation [10] and disinfection of microorganisms [11,12]. In recent years, many studies have been focused on TiO_2-based solar or longwave UV photocatalytic disinfection of different water matrices and demonstrated the success of this process. Of greater interest is the observation of a "residual disinfecting effect", which leads bacteria counts continue to decrease during the dark repair event, 24–60 h after the TiO_2-based photocatalytic treatment [13–15]. The underlying mechanisms responsible for the residual disinfecting effect have not been proved but are hypothesized to be associated partially with the oxidative damage of enzymes, cell walls and membranes, DNA and RNA by the ROS and their stable products [15]. The possessing of this residual disinfecting effect makes the TiO_2-based photocatalytic disinfection superior to photolytic UV-C disinfection that is known to allow bacteria to repair and regrowth. However, the generally higher UV-B fluences/doses (in an order of a few 10,000 mJ/cm^2) and TiO_2 dosages (250–1000 mg/L if in suspension) make the TiO_2 photocatalytic disinfection likely less attractive than the conventional UV-C disinfection at fluences/doses of 120 mJ/cm^2 or less in large-scale application. Another option of photocatalytic disinfection is using TiO_2 and UV-C in combination. Synergistic effects were observed in photocatalytic inactivation of *Cryptosporidium parvum* (*C. parvum*) using 1 mg/L TiO_2 (Degussa P25) in combination with LP UV irradiation at common doses (less than 40 mJ/cm^2) [16]. Higher TiO_2 doses have, however, detrimental effects on UV-C inactivation of *C. parvum* [16] and *E. coli* [17] and are not recommended, since TiO_2 particles absorb and scatter UV-C lights and protect microorganisms from irradiation. It is nevertheless unknown about whether the aforementioned residual disinfecting effect remains existing at TiO_2 dosages at mg/L level.

In this current study, we propose a new way to take advantages of both UV-C irradiation and photocatalytic oxidation processes and have experimentally verified that adding a small quantity of TiO_2 (Degussa P25, 1 mg/L in suspension or 1.44 mg/cm^2 immobilized on a glass substrate) during conventional UV-C irradiation can repress photoreactivation and dark repair of coliform bacteria. The repressive effects were observed consistently with variations of bacteria strains, temperature, salinities, UV sources and doses, and with/without nutrients. We have examined and suggest that such sub-lethal damage is likely associated with additional damage by the formed $^{\bullet}OH$ during the UV-TiO_2 irradiation and/or the production of a trace quantity of stable oxidants (primarily hydrogen peroxide (H_2O_2)); both of which are insufficient to enhance inactivation but are beneficial to control repairs of bacteria.

2. Experimental

2.1. Chemicals and solution preparation

All chemicals and nutrients used were of analytical grade and for R&D use, respectively, and dilutions were achieved with Maxima Ultra Pure water. Solutions and broths were stored at 4 °C and were brought back to room temperature before used.

2.2. Bacteria strains and growth procedures

The indigenous fecal coliform bacteria culture was isolated from local wastewater in Hong Kong. The isolation procedure consisted of a series of purification steps involving selecting a bacterial cell colony from a standard fecal coliform bacteria counting Petri-dish [18], transferring the colony to a tryptone–yeast-extract (TYE) broth (which contained 10 g/L tryptone, 5 g/L yeast extract, and 5 g/L sodium chloride), and sub-culturing the colony three times. *E. coli* (ATCC 11229 and ATCC 15597) were purchased from ATCC. Each pellet was re-suspended in 1 mL tryptone–soya-broth (TSB, Oxoid Ltd., Basingstoke, Hampshire, England) or ATCC medium 271, to revive the frozen cells. The bacterial culture used for experimental runs was freshly developed prior to experiments by transferring one loop of the culture stock to a sterilized glass tube of 20 mL appropriate broth and the mixture was then incubated at 37 °C for 18–24 h under continuous mixing. After propagation, the nutrient-rich bacterial suspension was centrifuged at 3000 rpm for 10 min to isolate the cell pellets from the broth, followed by supernatant removal and re-suspension with a phosphate buffer solution (PBS) (0.02 M, pH 7). This washing procedure was repeated three times to minimize the concentrations of non-cell-associated constituents in the solution. The bacterial concentration of the resultant suspension was approximately 10^9 CFU (colony forming unit) per mL. It was further diluted with the same PBS or testing water of specific matrix to yield a bacterial concentration of approximately 10^7 CFU/mL. All solutions and glassware used for the culture preparation were autoclaved at 121 °C and 1.5 kgf/cm^2 for 15 min prior to use.

2.3. Titanium dioxide coating

Glass substrate-immobilized TiO_2 was prepared by methods described in the literature [19–21] with slight modification. 1 g of TiO_2 powders (Degussa P25) were grinded in a mortar and pestle with 4 mL of highly diluted acetic acid (0.1 mL concentrated acetic acid added to 50 mL of water). A few drops of that were added each time and grinded to reach a suspension of smooth consistency. Few drops (approximately 0.5 mL) of Triton X-100 surfactants were then added and mixed. A microscope slide top glass (2.2 cm × 2.2 cm with an effective surface area of 4.76 cm^2) was taped on four sides to provide as a 40–50 μm spacer to control the thickness of the paste layer, and wiped cleaned with a tissue wet with ethanol. The TiO_2 mixture described above was pasted and disturbed uniformly by sliding a glass rod over the plate. The paste was air dried for 1 min, followed by removing the tapes carefully without scratching the TiO_2 coating. The glass was heated on a hotplate at approximate 400 °C in a hood for 10–20 min until the color changed from white to brown and finally back to white. The TiO_2-coated glass was then allowed to cool down slowly to room temperature. The amount of TiO_2 on the coating plate was 6.85 ± 0.20 mg, corresponding to 1.44 ± 0.04 mg/cm^2.

2.4. UV and fluorescence light irradiation

Collimated beam apparatuses consisting of either two LP UV lamps (254 nm, UVP, Upland) or one MP UV lamp (220–580 nm, UVV-5, Hanovia) were used as the UV sources. The LP UV lamps

were placed horizontally in a polyvinyl chloride box, and a polyvinyl chloride collimating tube (inner diameter of 5.5 cm and length of 30 cm) was extended from the center-bottom of the horizontal box to create collimated, parallel UV lights. Proper heat ventilation was provided. The intensity of the collimated irradiation from the LP UV lamps was measured at the point of exposure by a radiometer (DRC-100X, Spectronics Corporation) and the value was 0.2 mW/cm^2 unless specified elsewhere. The MP UV collimated beam apparatus was arranged in the same way as the LP UV one except that the housing was made of stainless steel and the length of the collimating tube was 60 cm. The time-dependent doses of MP UV were determined by biodosimetry using the target bacteria. In short, two 7 mL fresh bacteria stir-mixed testing solutions were placed on two dishes with an internal diameter of 30 mm and water depth of 9 mm. One solution was exposed to the collimated LP UV unit and the other was exposed to the collimated MP UV unit. Samples from both dishes were taken at different exposure time to determine the time–response curves for both exposures. The corresponding irradiation time for MP UV to reach the same inactivation was then determined.

The fluorescence light irradiation was conducted with two 36 W fluorescent lamps (Philips Lighting Co.) and the light was further filtered with a plastic cover. The wavelength-specific intensity measured at the sample surface underneath the plastic cover by a spectroradiometer (RPS900-R, International Light) was shown in Fig. 1. The UV–vis spectrum scan shows that the filtered fluorescence lights are in the wavelength range of 400–700 nm only.

2.5. Experimental procedures for inactivation/reactivation tests

After the UV apparatuses were turned on at least half an hour prior to examination to ensure a uniform UV intensity output, aqueous suspensions of specific bacteria produced as aforementioned were subjected to UV irradiation, with or without TiO_2. For experiments with additions of TiO_2 in suspension, given aliquots of an autoclaved and ultrasound-dispersed TiO_2 (Degussa P25) stock solution were added into testing solutions to make 1 mg/L (unless specified elsewhere) prior to UV irradiation. Similarly, for experiments with additions of attached TiO_2, the TiO_2 coating plate prepared above was sterilized and placed at the bottom of a dish with the coating on top. The UV doses/fluences were controlled by varying the irradiation time, which was achieved by manually blocking the end of the collimating tube with a sheet of aluminum film. The irradiation time was measured with a stopwatch and the UV doses (in mJ/cm^2) were calculated as the product of the measured UV intensity and the irradiation time. Samples (0.5 mL) were collected before and after the UV exposure and subjected to bacteria numeration by dilution and membrane filtration techniques [18]. The UV-irradiated solutions were then either kept under the filtered fluorescence light to allow photoreactivation or in the dark to allow dark repair to take place. Samples were periodically taken at given time intervals for a total period of 3 h for photoreactivation and 24 h for dark repair and subjected to bacteria numeration as aforementioned. It should be noted that TiO_2 in suspension was not removed from the solution after UV irradiation. On the other hand, the TiO_2-coated plate was removed immediately after UV-C exposure and the residual TiO_2 concentration in the irradiated solution, after the plate was removed, was less than 0.1 mg/L, which is the limit of detection using a UV–vis spectrometer (Lambda 25, PerkinElmer). All experiments were replicated at pH 7 and 20 ± 2 °C, except where specifically indicated.

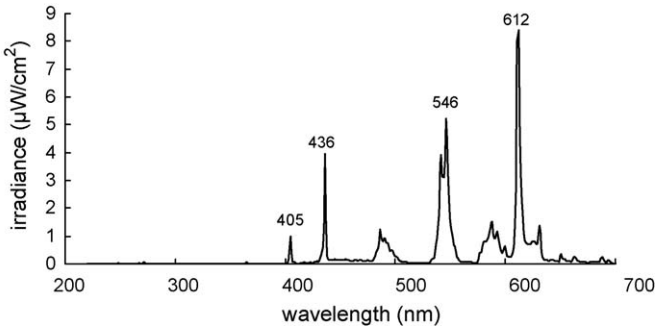

Fig. 1. UV–vis intensity spectrum of the fluorescence light measured at the sample surface underneath a plastic cover.

2.6. Oxidant analysis and additional oxidant production tests

Concentrations of •OH in testing solutions were estimated according to Cho et al. [11] using the common probing compound *para*-chlorobenzoic acid. The DPD colorimetric method [18] with potassium iodide additions was used to determine concentrations of total residual oxidants (normalized to equivalent H_2O_2 molar concentrations). Concentrations of H_2O_2 in the illuminated solutions were estimated colorimetrically with the titanium-4-(2′-pyridylazo) resorcinol (Ti-PAR) reagent, according to Matsubara et al. [22] and Kikuchi et al. [23]. In brief, 4 mL of the filtered illuminated sample was added to 4 mL of the Ti-PAR reagent and 1 mL of the buffer solution was added to adjust the pH to around 8.6. After the mixed solution was warmed at 45 °C for 2 h, absorbance at 508 nm was measured and compared to the calibration curve [22,23].

Tests for production of stable oxidants were conducted by illuminating a PBS solution of different TiO_2 concentrations and salinity levels with UV-C light for 30–40 min. The prolonged irradiation time of 30–40 min was chosen to yield detectable oxidant concentrations. Other testing conditions remained similar to that of the inactivation tests. The illuminated solution was sat for several minutes to allow the depletion of short-life radicals, filtered through a 0.45-μm membrane, and subjected to analyses of total residual stable oxidants and H_2O_2.

3. Results and discussion

3.1. Post-UV photoreactivation and dark repair of bacteria in the absence and presence of TiO_2

The extent of post-UV photoreactivation after the UV and UV-TiO_2 irradiation was evaluated after bacteria suspensions, including *E. coli* (ATCC 15597 and ATCC 11229) and locally isolated fecal coliform bacteria were UV irradiated at an average dose of 12 mJ/cm^2 under the condition provided in the caption of Fig. 2. Data presented at time zero here and thereafter represent the bacteria counts right after the completion of UV or UV-TiO_2 exposure and before the occurrence of photoreactivation. The average UV doses reported here and later were calculated by accounting for the incident fluence, absorbance within the bacteria suspension and reflection at the air/water interface.

As shown in Fig. 2, in the absence of TiO_2 during UV exposure, photoreactivation of these three coliform bacteria occurred under fluorescence lights and the bacteria counts increased with increasing fluorescence light illumination time. The maximum bacteria counts were reached within 1–2 h and were approximately 20 times higher than that obtained right after UV irradiation. The results agree with most data reported in the literature. However, with the presence of 1 mg/L TiO_2 in suspension during UV exposure, photoreactivation of these

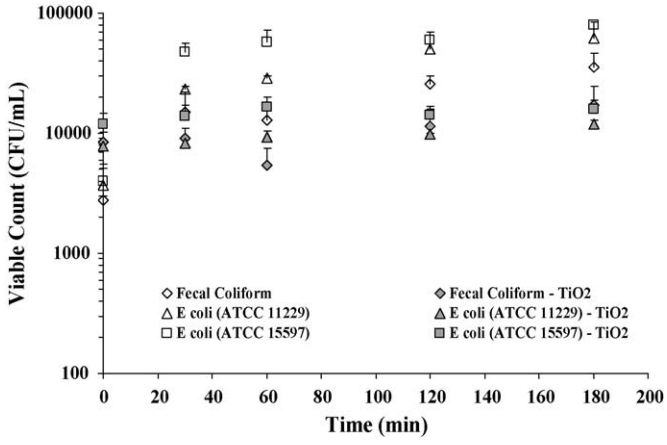

Fig. 2. Time-dependent viable counts of coliform bacteria (CFU/mL) after LP UV irradiation alone (open symbols) and LP UV-TiO_2 (in suspension) irradiation (filled symbols) followed by photoreactivation under fluorescence light up to 3 h at 0.016% salinity, 22 °C and pH 7. UV dose = 12 mJ/cm^2 and TiO_2 concentration = 1 mg/L, if present.

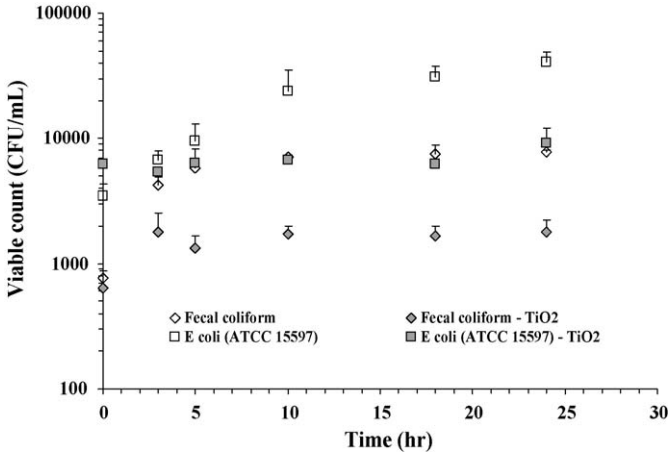

Fig. 3. Time-dependent viable count of coliform bacteria (CFU/mL) after LP UV irradiation alone (open symbols) and LP UV-TiO_2 (in suspension) irradiation (filled symbols) followed by dark repair up to 24 h at 3.5% salinity, 22 °C and pH 7. UV dose = 12 mJ/cm^2 and TiO_2 concentration = 1 mg/L, if present.

bacteria under fluorescence lights was significantly repressed, giving insignificant increases in bacteria counts of less than two times and within the measurement errors (indicated by the error bar in Fig. 2, which displays the difference between the mean and one of the measured data of the two duplicated samples). It should be also noted that the presence of 1 mg/L TiO_2 somewhat reduces the disinfection efficiency of UV irradiation, giving a higher bacteria counts right after the UV irradiation. This detrimental effect on UV-C disinfection has been reported and is attributed to the light screening by the TiO_2 particles in solution [17]. We here recorded 9 mm UV transmittance of 92% in 1 mg/L TiO_2 suspension. Enhancing bacteria inactivation was always observed, on the other hand, when TiO_2 was in combination with solar, UV-A, or UV-B irradiation [11,24,25]. It is obvious because photocatalytic disinfection at wavelengths of light higher than the UV-C range relies on the photocatalytically generated •OH and photolysis with lights at those ranges cannot provide good disinfection. On the other hand, the generation of •OH and its disinfection is minor under the conditions used in current study, which is further discussed in Section 3.2.

As shown in Fig. 3, in the absence of TiO_2 during UV irradiation, bacteria repaired but the dark repair took a much longer time than photoreactivation did to reach the plateau of approximate 12-fold increases, compared to the bacteria counts at the end of UV exposure. Post-UV dark repair of coliform bacteria was also repressed by the provision of 1 mg/L TiO_2 in suspension during the UV exposure.

3.2. Formation of oxidants during and after UV-TiO_2 irradiation

Oxidants, including •OH, total stable oxidants and H_2O_2, were measured to explore possible explanation for the post-UV repressive effect by provision of 1 mg/L TiO_2 in suspension. Using the probing technique as described above, the •OH concentration in the bacteria suspension during the UV-TiO_2 exposure was found to be approximately 3×10^{-14} M. This concentration is insufficient to exert notable coliform inactivation, using the linear correlation coefficient between inactivation of *E. coli* and •OH concentrations reported in Cho et al. [11], in agreement with the experimental observation in the current study. However, it is unknown, though possible, whether the exposure to this low concentration of •OH during UV-TiO_2 irradiation can create some sub-lethal damages (e.g., deleterious effects on enzymes responsible for repair, blockage of the enzymes' synthesis, etc. [15]) to repress the subsequent bacteria repair. The repressive effect was not associated with TiO_2-based photocatalytic disinfection during the post-UV event, since the filtered fluorescence lights (at wavelengths from 400 to 700 nm) cannot excite the TiO_2 (Degussa P25) to form •OH. Hydroxyl radicals were not detectable in the post-UV event and in our control with no UV illumination.

Another way to explain the repression is from the angle of the "residual disinfecting effect" by stable oxidants such as H_2O_2 after the alternation of bacterial cell membrane permeability [13–15]. Additional tests were performed to determine the possible formation of stable residual oxidants in TiO_2-containing solutions after UV illumination. The illumination time here was extended to 30–40 min to obtain measurable oxidant concentrations. As shown in Table 1, illuminated TiO_2 solutions buffered at pH 7 contained stable oxidants in sub-μM level and the concentrations were independent to the salinity of the solutions. The concentrations of stable oxidants increased with increasing TiO_2 concentration (Fig. 4) and these oxidants could persist in water for a few hours (Fig. 4 insert) under the fluorescent light. Fig. 4 also shows the measured concentrations of H_2O_2, which are in good agreement with those of the total stable oxidants, indicating that H_2O_2 can be the primary oxidant produced. The results suggest that the stable oxidant/H_2O_2 concentrations in the UV-TiO_2 events employed in our disinfection tests shall be around 0.02–0.03 μM. No stable oxidant or H_2O_2 was measured in the controls of no UV illumination or no TiO_2 addition (Table 1).

Total oxidant/H_2O_2 of 0.02–0.03 μM is unlikely to exert lethal bactericidal effects, but may perturb regular bacteria metabolic activity by causing some redox reactions inside the bacteria cells and subsequently repress bacteria repair. It has been reported by Bin Alam et al. [26] that H_2O_2 generation in μM level was quite correlated with the long-lasting residual effect on algal growth control by UV-irradiation. It was proposed that H_2O_2 might lead to the production of other reactive short-lived species and thus gave the residual effect. Kikuchi et al. [23] have proposed that the long-lasting bactericidal effect of low to sub-μM levels of H_2O_2, which was lower than that needed to kill bacteria, may be attributable to its reactions with other oxygen species in photocatalytic disinfection using TiO_2 thin film. In our system, similar mechanism may be implicated in the repair repression process.

Table 1
Concentrations of total stable oxidants under different solution compositions.

Solution composition	UV illumination time (min)	Total stable oxidants (μM)
1 mg/L TiO_2 + 0.02 M phosphate buffer	30	0.53 ± 0.03
1 mg/L TiO_2 + 0.02 M phosphate buffer + 0.016% salinity	30	0.58 ± 0.03
1 mg/L TiO_2 + 0.02 M phosphate buffer + 0.9% salinity	30	0.66 ± 0.04
1 mg/L TiO_2 + 0.02 M phosphate buffer + 3.5% salinity	30	0.69 ± 0.01
0.02 M phosphate buffer + 0.016% salinity	30	ND
1 mg/L TiO_2 + 0.02 M phosphate buffer + 0.016% salinity	0	ND

ND, not detectable.

Last, the UV-TiO_2 exposure may induce additional irreparable damage other than formation of dimers. However, we are neither able to verify this hypothesis nor the hypothesis in enzymatic damage previously mentioned. Besides, the hypotheses associated with oxidative lethal damage to bacteria cells proposed in Rincón and Pulgarin [15] may not be suitable here, because of the different killing mechanisms during illumination. UV-C induced DNA damage is the major lethal effect on bacteria in our current study; while photocatalytic generation of •OH is the major one in the literature where dark repair are examined [13–15].

3.3. Effects of environmental conditions on the repressive effect

We also evaluated the effects of environmental conditions, such as TiO_2 concentrations and phases, temperature, matrix compositions including salt and nutrient contents, and UV sources, intensities and doses, on the repression of bacteria repair by the TiO_2-modified UV-C disinfection.

3.3.1. Effects of TiO_2 concentrations and phases

Fig. 5a illustrates the effects of TiO_2 concentrations on UV-C inactivation and post-UV photoreactivation of coliform bacteria. Increasing the TiO_2 concentration to 10 mg/L in suspension during UV exposure completely stopped the subsequent photoreactivation of coliform bacteria; however, inactivation efficiency right after UV exposure was also significantly reduced. As discussed above and also in the literature [17], higher concentrations of TiO_2 particles give severer detrimental, screening effects on UV-C disinfection (9 mm UV transmittance of 92 and 46% at 1 and 10 mg/L TiO_2, respectively). These results show that TiO_2 particles of higher concentration absorb more lights to produce higher quantities of oxidants that are sufficient for better repression of bacteria repair but yet not enough for accomplishing inactivation. The finding indicates the necessity of controlling TiO_2 dosages in suspension to achieve both inactivation and repression of reactivation.

To eliminate the detrimental effect of TiO_2 concentrations on inactivation, TiO_2 particles immobilized to a substrate were used to replace the suspended TiO_2 and its effectiveness on repressing bacteria repair was investigated. Immobilizing TiO_2 on a substrate also eliminates problems associated with collection of the catalysts after treatment. This approach also allows us to verify the importance of the presence of TiO_2 during the post-UV repair event. UV irradiation with immobilized TiO_2 gave the same log-inactivation as that in the absence of TiO_2 and repressed the photoreactivation of coliform bacteria (Fig. 5b). Approximately 2×10^{-13} M •OH was estimated during UV irradiation of the TiO_2

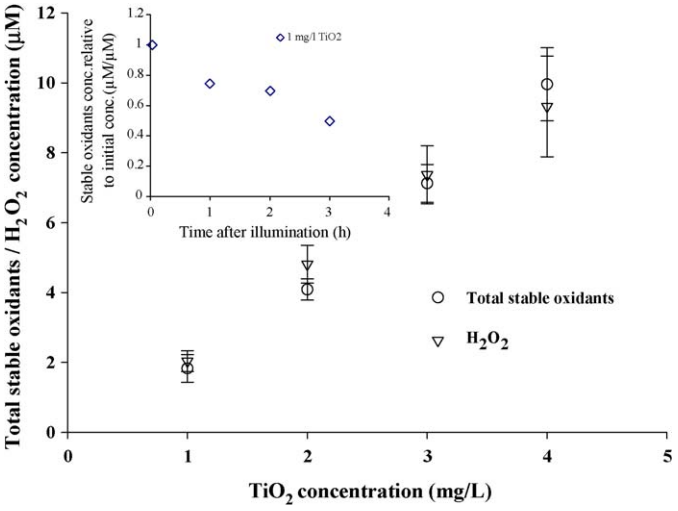

Fig. 4. Total stable oxidants and H_2O_2 production after 40 min UV-C illumination of solutions of different concentrations of TiO_2 particles at 0.016% salinity. UV intensity = 0.1 mW/cm^2 (insert: time-dependent decay of total stable oxidants after removal of the solution from LP UV-TiO_2 exposure).

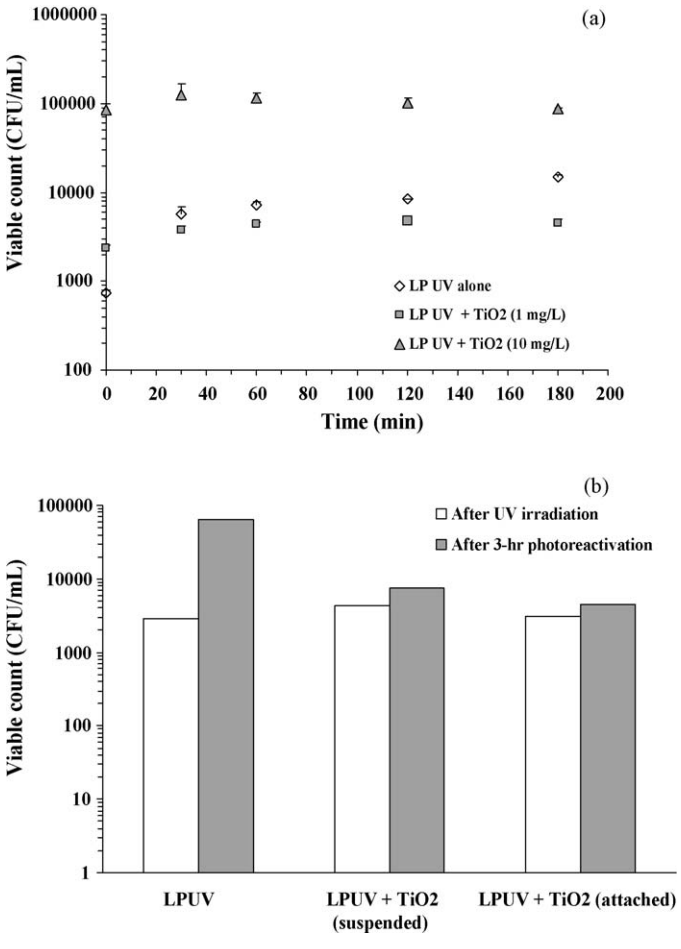

Fig. 5. Effects of (a) TiO_2 concentration and (b) phases of TiO_2 on viable counts of fecal coliform bacteria (CFU/mL) after LP UV irradiation alone and LP UV-TiO_2 irradiation followed by photoreactivation under fluorescence light up to 3 h at 3.5% salinity, 22 °C and pH 7. UV dose = 12 mJ/cm^2 and TiO_2 concentration = 1 mg/L (in suspension) and 1.44 mg/cm^2 (in attachment), if present.

plate and this concentration of •OH could not give notable coliform bacteria inactivation [11]. The log-inactivation was not associated with the adsorption of coliform bacteria onto the TiO_2 surface. Tests were conducted with the same bacteria suspensions treated with 1 mg/L TiO_2 particles or the sterilized TiO_2 plate in the dark and under fluorescent light and controls of no addition of TiO_2. Data showed the same bacteria counts in the testing solutions and the controls and no change of bacteria counts for up to 3 h. Thus, under the testing condition, the inactivation is mainly attributed to the UV-C irradiation and is not affected by the presence of the TiO_2 plate. The results also indicate that the post-UV repressive effect does not require the presence of TiO_2 during the post-UV event because the remaining TiO_2 concentration was below the limit of detection (0.1 mg/L) after plate removal. Therefore, UV-C irradiation with immobilized TiO_2 is the preferable choice to achieve repression of bacteria repair without compromising the effectiveness of UV-C disinfection.

3.3.2. Effect of temperature

As shown in Fig. 6, variations in temperature from 10 to 35 °C did not affect UV inactivation but the extent of photoreactivation, in the absence of TiO_2, was relatively less at 10 °C. This is in accordance with the literature finding that low temperature can, to some extent, reduce photoreactivation and dark repair [7]. Still, the provision of TiO_2, 1 mg/L in suspension, repressed the photoreactivation of coliform bacteria at all the temperatures tested. This suggests that temperature does not have obvious impact on repression of bacteria photoreactivation by the TiO_2-modified UV-C disinfection.

3.3.3. Effect of salt and nutrient contents

As shown in Fig. 7a, in the absence of TiO_2, photoreactivation was found to be less at salinity of 0.016% where the inactivation by UV alone was less. On the other hand, the repression of bacteria photoreactivation by providing TiO_2 during UV exposure persisted, independent of the salinity level.

Fig. 7b shows the effect of the presence of nutrient (tryptone–soya-broth) on bacteria photoreactivation in the absence and presence of TiO_2. In the absence of TiO_2, provision of the nutrient gave higher bacteria concentrations after 3 h fluorescence light exposure, which was attributable to both bacteria photoreactivation and regrowth that could not be differentiated. Nevertheless, the repressive effect on bacteria photoreactivation after the UV-

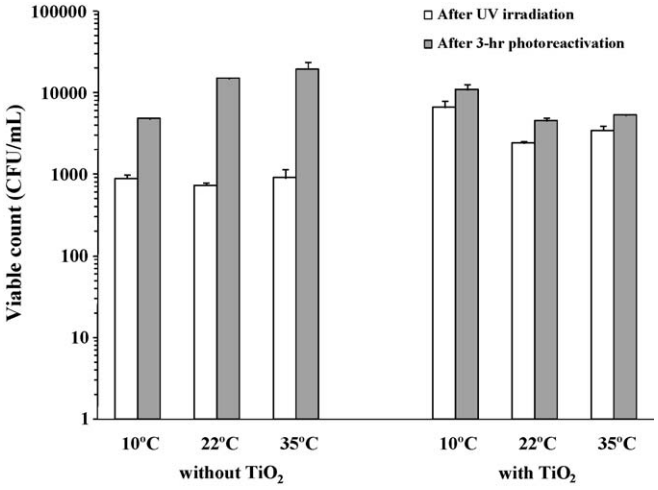

Fig. 6. Effect of temperature on viable counts of fecal coliform bacteria (CFU/mL) after LP UV irradiation alone and LP UV-TiO_2 irradiation followed by 3 h photoreactivation under fluorescent light at 0.016% salinity and pH 7. UV dose = 12 mJ/cm^2 and TiO_2 concentration = 1 mg/L, if present.

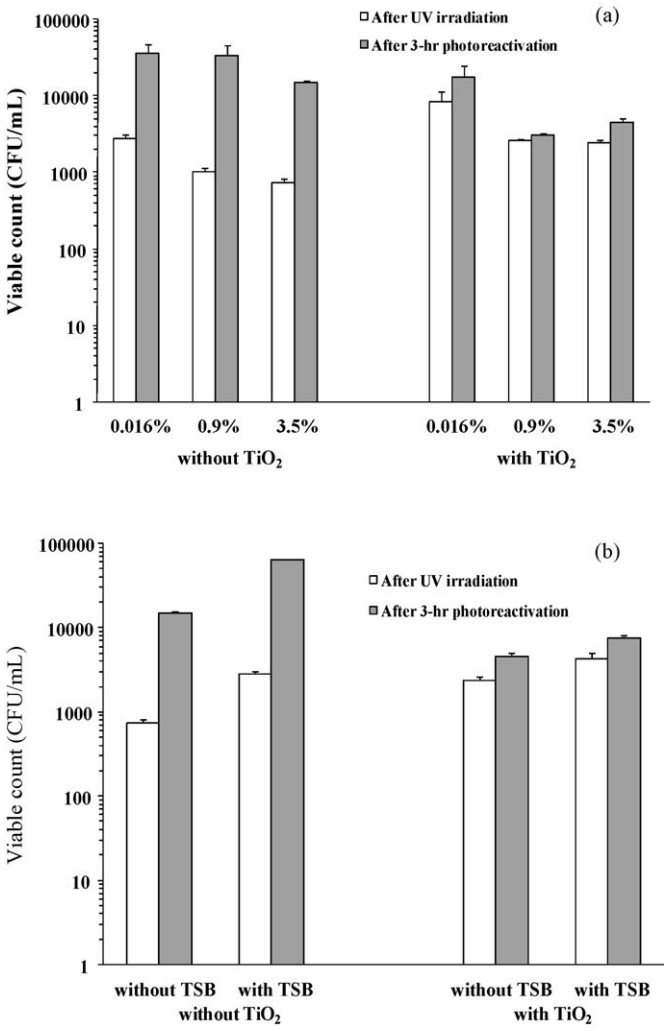

Fig. 7. Effects of (a) salinity and (b) nutrient (TSB) on viable counts of fecal coliform bacteria (CFU/mL) after LP UV irradiation alone and LP UV-TiO_2 irradiation followed by 3 h photoreactivation under fluorescent light at 22 °C and pH 7. UV dose = 12 mJ/cm^2 and TiO_2 concentration = 1 mg/L, if present.

TiO_2 exposure is not affected by the presence of the TSB nutrient, indicating the good applicability of this approach in handling real-world samples.

3.3.4. Effect of UV sources, doses and intensities

To investigate the dependency of the repression of photoreactivation by the UV-TiO_2 exposure on UV doses, intensity and sources, we conducted tests following an orthogonal matrix experimental design by changing one parameter at a time from the baseline condition, which was UV dose of 12 mJ/cm^2, UV intensity of 0.2 mW/cm^2 and LP UV. Variable parameters included UV doses of 8, 12 and 16 mJ/cm^2; UV intensities of 0.12 and 0.2 mW/cm^2; and UV sources of LP UV and MP UV.

As shown in Fig. 8, both situations (in the absence and presence of TiO_2) share the common trend of increasing inactivation efficiency with increasing UV dose from 8 to 16 mJ/cm^2 at a constant UV intensity. Adding TiO_2 during UV irradiation, nevertheless, repressed the subsequent photoreactivation and gave insignificant increases of bacteria counts after 3 h under fluorescence lights. The results indicate that a UV dose of 8 mJ/cm^2 is sufficient to create the sub-lethal damage by the UV-TiO_2 exposure to repress the reactivation. The extent of photoreactivation remained similar at the same UV dose (12 mJ/cm^2) but different UV intensities (0.12 and 0.2 mW/cm^2) in the absence of TiO_2.

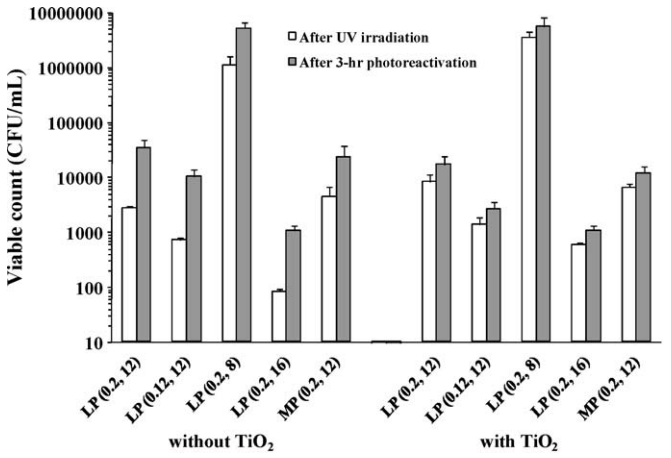

Fig. 8. Effects of UV doses, intensities and sources on viable counts of fecal coliform bacteria (CFU/mL) after LP UV irradiation alone and LP UV-TiO_2 (in suspension) irradiation followed by 3 h photoreactivation under fluorescent light at 0.016% salinity, 22 °C and pH 7. TiO_2 concentration = 1 mg/L, if present. UV intensity = 0.12 and 0.2 mW/cm^2 as indicated in the first number in brackets, UV dose = 8, 12 and 16 mJ/cm^2 as indicated in the second number in brackets, UV sources, LP and MP UV, as indicated outside brackets.

Nevertheless, the repressive effects of the UV-TiO_2 exposure remained similar, although the irradiation time varied at different UV intensity to give the same UV dose.

MPUV irradiation at equivalent doses resulted in comparatively less photoreactivation of coliform bacteria than LP UV did in the absence of TiO_2. We did not observe complete elimination of bacteria photoreactivation by MPUV that has been reported in the literature [27] and the 5-fold increase in bacteria counts after photoreactivation still needs to be controlled. Adding TiO_2 during MP UV irradiation further repressed the bacteria reactivation.

4. Conclusions

The repression of post-UV photoreactivation and dark repair of coliform bacteria by the provision of TiO_2 at very low concentrations in suspension or in the immobilized phase during UV-C disinfection promises and secures a range of stand-alone UV applications to pathogen controls in water, wastewater and aquacultural industries. Such a modified UV technique with immobilized TiO_2 can be easily implemented in existing UV devices by coating some TiO_2 particles on quartz jackets surrounding UV lamp tubes and/or on inner surfaces of irradiators/contactors. No other modification is required; because the UV-C irradiation remains to provide lethal effects to inactivate bacteria, while the provision of TiO_2 is not for inactivation but for delivering the sub-lethal repressive effect. It should be reemphasized that the application of TiO_2 and UV for the present propose is different from photocatalytic disinfection. The latter requires much higher TiO_2 concentrations and UV doses/fluences and relies primarily on the generation of $^{\bullet}OH$ and other reactive oxygen species to give the residual disinfecting effect. The former, although the actual mechanisms are not fully ascertained, possibly utilizes a much smaller quantity of TiO_2 and common UV-C doses/fluences to create some sub-lethal damages and/or produce stable residual oxidants (H_2O_2 in majority) that contribute in part to the repression of bacteria repair and reactivation. The repression has found to occur under all conditions testes and does not require the presence of TiO_2 in the post-UV event. However, it should be noted that this study was conducted in mixed-batch reactors in the laboratory.

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